FQNs (Fluoroquinolones) ELISA Kit

Catalog No: E-FS-E166

96T

Version Number:	V1.4
Replace version:	V1.3
Revision Date:	2025.09.01

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Toll-free: 1-888-852-8623 Tel: 1-832-243-6086 Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Test principle

This kit uses Competitive-ELISA as the method for the quantitative detection. It can detect Fluoroquinolones (FQNs) in samples, such as milk, muscle, egg etc. This kit is composed of ELISA Microtiter plate, HRP conjugate, antibody working solution, standard and other supplementary reagents. The microtiter plate in this kit has been pre-coated with coupled antigen. During the reaction, FQNs in the samples or standard competes with coupled antigen on the solid phase supporter for sites of anti-FQNs antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each microtiter plate well, and substrate reagent is added for color development. There is a negative correlation between the OD value of samples and the concentration of FQNs. The concentration of FQNs in the samples can be calculated by comparing the OD of the samples to the standard curve.

Technical indicator

Reaction mode(Incubation time and temperature)**:** 25℃;30 min, 15min.

Detection limit: Muscle (Fish, Shrimp-Method 1) ---1 ppb; Muscle (Chicken, Duck, Pork, Beef, Lamb -Method 1) ---2 ppb; Muscle (Chicken, Duck -Method 2) ---5 ppb; Muscle (Pork, Beef, Lamb-Method 2), Milk, Milk powder, Eggs, Bovine serum ---10 ppb; Liver, Urine---30 ppb.

Cross-reactivity:

Enrofloxacin	97%
Norfloxacin	115%
Ciprofloxacin	100%
Lomefloxacin	119%
Flumequine	107%
Peflacine	132%
Danofloxacin	127%
Enoxacin	98%
Ofloxacin	38%
Oxolinic acid	181%
Marbofloxacin	108%

Sample recovery rate: 90%±30%.

Kits components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	1.5 mL each (ppb=ng/mL=ng/g) (0 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb, 24.3 ppb)
HRP Conjugate	7 mL
Antibody Working Solution	7 mL
Substrate Reagent A	7 mL
Substrate Reagent B	7 mL
Stop Solution	7 mL
20×Concentrated Wash Buffer	25 mL
20×Concentrated Sample Diluent	50 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

Other materials required but not supplied

Instrument: Microplate reader, Printer, Homogenizer, Vortex mixer, Nitrogen evaporators, Water bath, Centrifuge, Graduated pipette, Balance (sensibility 0.01g).

Micropipette: Single channel (20-200 μL, 100-1000 μL), Multichannel (30-300 μL).

Reagents: Acetonitrile, N-hexane, HCl.

Notes

1. The overall OD value will be lower when reagents have not been brought to room temperature before use or room temperature is below 25℃.
2. If the wells turn dry during the washing procedure, it will lead to bad linear standard curve and poor repeatability. Operate the next step immediately after wash.
3. Mix thoroughly and wash the plate completely. The consistency of wash procedure can strongly affect the reproducibility of this ELISA kit.
4. FOR RESEARCH USE ONLY. ELISA Microtiter plate should be covered by plate sealer. Avoid th e kit to strong light.
5. **Each reagent is optimized for use in the E-FS-E166. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-FS-E166 with different lot numbers.**
6. Substrate Reagent should be abandoned if it turns blue color. When OD value of standard (concentration: 0) < 0.8 unit (A450nm < 0.8), it indicates the reagent may be deteriorated.

7. Stop solution is caustic, avoid contact with skin and eyes.
8. As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test.
9. Even the same operator might get different results in two separate experiments. In order to get reproducible results, the operation of every step in the assay should be controlled.
10. After adding the sample diluent and acetonitrile, vortexize immediately to make the tissue as dispersed as possible, which directly affects the test results.
11. If the **20×Concentrated Sample Diluent** precipitates crystallization, it needs to be warmed at room temperature and can be used until the crystallization dissolves.
12. Please use fresh samples for testing.
13. **For mentioned sample fast and efficient extraction methods are included in the kit description. Please consult technical support for the applicability if other sample need to be tested.**
14. The kit is used for rapid screening of actual samples. If the test result is positive, the instrument method such as HPLC, LC/MS, etc. can be used for quantitative confirmation.

Storage and expiry date

Store the kit at 2-8℃. Do not freeze any test kit components.

Return any unused microwells to their original foil bag and reseal them together with the desiccant provided and further store at 2-8℃.

Expiry date: expiration date is on the packing box.

Experimental preparation

Restore all reagents and samples to room temperature before use.

Open the microplate reader in advance, preheat the instrument, and set the testing parameters.

1. Sample pretreatment Notice:

Experimental apparatus should be clean, and the pipette should be disposable to avoid cross-contamination during the experiment.

2. Solution preparation

Please prepare solution according to the number of samples. Don't use up all components in the kit at once!

Solution 1: 0.2 M HCl Solution

Dilute 1.72 mL of **HCl** to 100 mL with deionized water, mix fully.

Solution 2: Sample Diluent A

Dilute **20×Concentrated Sample Diluent** with deionized water. (20×Concentrated Sample Diluent (V): Deionized water (V) = 1:9).

Solution 3: Sample Diluent B

Take 100mL **Sample diluent A**, add 1g NaCl solid, mix well and set aside.

Solution 4: Wash Buffer

Dilute **20×Concentrated Wash Buffer** with deionized water. (20×Concentrated Wash

Buffer (V): Deionized water (V) = 1:19).

3. Sample pretreatment procedure

3.1 Pretreatment of Muscle(Fish, shrimp, Chicken, duck, pork, beef, lamb) sample(Method 1):

(1) Remove fat from sample. Homogenize the representative sample with a homogenizer and mix fully.

(2) Weigh 1±0.05 g of muscle homogenate into a 15 mL centrifuge tube.

(3) **For Fish, shrimp:**
Add 0.5 mL of **20×Concentrated Sample Diluent** and vortex for 30s.
For Chicken, duck, pork, beef, lamb:
Add 0.5 mL of **0.2 M HCl Solution** (Solution 1) and vortex for 30s.

(4) Add 4.5 mL **Acetonitrile**, vortex immediately for 2 min until the tissue is completely dispersed. Centrifuge for 5 min at 4000 g at room temperature.

(5) Remove 3 mL of the clear upper organic layer solution to a new centrifuge tube, dry at 50-60℃ with nitrogen evaporators or water bath. (Please do it in a ventilated environment.)

(6) Add 2 mL of **N-hexane** and vortex for 30s. Then add 1 mL of **Sample Diluent A** (Solution 2) and vortex for 30 s to mix fully. Centrifuge for 5 min at 4000 rpm at room temperature.

(7) Completely eliminate the upper layer of n-hexane and the impurities in the middle layer;

(8) Take 50 µL of solution for analysis.

Note: Sample dilution factor: 2, detection limit: 1 ppb (Fish, shrimp); 2 ppb (Chicken, duck, pork, beef, lamb)

3.2 Pretreatment of Muscle(Chicken, duck, pork, beef, lamb) sample(Method 2):

(1) Remove fat from sample. Homogenize the representative sample with a homogenizer and mix fully.

(2) Weigh 1±0.05 g of muscle homogenate into a 50 mL centrifuge tube.

(3) Add 9.5mL deionized water and 0.5mL **20×Concentrated Sample Diluent** successively, Vortex immediately for 1 min until the tissue is completely dispersed.

(4) Then centrifuge at 4000 g for 10 min at room temperature.

(5) Immediately take 50µL of supernatant for detection.

Note: Sample dilution factor: 10, detection limit: 5 ppb (Chicken, duck); 10 ppb (pig, cow, sheep)

3.3 Pretreatment of Milk powder sample:

(1) Weigh 1±0.05g milk powder sample, add 10 mL deionized water, swirl fully for 1 min, take 100µL sample in the centrifuge tube.

(2) Add 900 µL **Sample Diluent B (**Solution 3**),** Vortex for 30s.

(3) Take 50 µL for analysis.

Note: Sample dilution factor: 10, detection limit: 10 ppb

3.4 Pretreatment of Raw milk, Bovine serum, Egg sample:

(1) Pipette 100 µL of the homogenized egg sample or 100 µL raw milk, bovine serum into a centrifuge tube.

(2) Add 900 µL **Sample Diluent B** (Solution 3), vortex for 30s.

(3) Take 50 µL for analysis.

Note: Sample dilution factor: 10, detection limit: 10 ppb

3.5 Pretreatment of Pig liver sample:

(1) Weigh 1±0.05 g of muscle homogenate into a 15 mL centrifuge tube.

(2) Add 9.5mL of deionized water, 0.5 mL of **0.2 M HCl Solution** (Solution 1) in sequential and vortex for 1 min until the tissue is completely dispersed.

(3) Centrifuge for 5 min at 4000 g at room temperature.

(4) Take 200 µL of supernatant into a new centrifuge tube, add 200 µL **Sample Diluent A** (Solution 2) and 200 µL deionized water, vortex for 30s.

(5) Take 50 µL of solution for analysis.

Note: Sample dilution factor: 30, detection limit: 30 ppb

3.6 Pretreatment of Pig urine sample:

(1) Pipette 100 µL of the urine sample into a centrifuge tube.

(2) Add 900 µL **Sample Diluent A** (Solution 2), vortex for 30s.

(3) Take 50 µL for analysis.

Note: Sample dilution factor: 10, detection limit: 30 ppb

Assay procedure

Restore all reagents and samples to room temperature (25℃) before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2-8℃.

1. **Number:** number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells. **Standard and Samples need test in duplicate.**
2. **Add Sample:** add 50 µL of **Standard** or **Sample** per well, then add 50 µL of **HRP Conjugate** to each well and 50 µL of **Antibody Working Solution** in sequential. Gently oscillate for 5 s to mix thoroughly and cover the plate with plate sealer. Incubate at 25℃ for 30 min in shading light.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 260 µL of **Wash Buffer** (Solution 4) to each well and wash. Repeat the wash procedure for 5 times, 30 s intervals/time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
4. **Color Development:** add 100 µL of **Substrate Reagent A** and **Substrate Reagent B** mixture. (**Substrate Reagent A** and **Substrate Reagent B** are mixed 1:1 according to volume, must be fully mixed, the mixture is used within 5 minutes, avoid the use of metal container, avoid stirring reagents.) Gently oscillate for 5 s to mix thoroughly. Incubate 25℃ for 15-20 min at in shading

light.

5. **Stop Reaction:** add 50 μL of **Stop Solution** to each well, oscillate gently to mix thoroughly.
6. **OD Measurement:** determine the optical density (OD value) of each well at 450 nm (reference wavelength 630 nm) with a microplate reader. This step should be finished in 5 min after stop reaction.

Result analysis

1. **Absorbance(%)=A/A_0×100%**

   A: Average absorbance of standard or sample

   A_0: Average absorbance of 0 ppb Standard

2. **Drawing and calculation of standard curve**

   Create a standard curve by plotting the absorbance percentage of each standard on the y-axis against the log concentration on the x-axis to draw a semi-logarithmic plot. Add average absorbance value of sample to standard curve to get corresponding concentration. **If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor.**

   For this kit, it is more convenient to use professional analysis form for accurate and fast analysis of batch samples.

Fluoroquinolones (E-FS-E166) **Standard Curve**